Expert Opinion

Ashley Publications
www.ashley-pub.com

Peptides, Proteins & Antisense

Antisense therapy for restenosis following percutaneous coronary intervention

Nicholas Kipshidze†, Mykola Tsapenko, Patrick Iversen & Denis Burger
†*Lenox Hill Hospital, Department of Interventional Cardiac & Vascular Services, 130 East 77th Street, New York, NY 10021, USA*

Recent advances in vascular gene transfer have shown potential new treatment modalities for cardiovascular disease, particularly in the treatment of vascular restenosis. The antisense approach to inhibiting gene expression involves introducing oligonucleotides complementary to mRNA into cells in order to block any one of the following processes: uncoiling of DNA, transcription of DNA, export of RNA, DNA splicing, RNA stability, or RNA translation involved in the synthesis of proteins in cellular proliferation. The approach includes the use of antisense oligonucleotides, antisense mRNA, autocatalytic ribozymes, and the insertion of a section of DNA to form a triple helix. Proof of principle has been established that inhibition of several cellular proto-oncogenes, including DNA binding protein c-myb, non-muscle myosin heavy chain, PCNA proliferating-cell nuclear antigen, platelet-derived growth factor, basic fibroblast growth factor and c-myc, inhibits smooth muscle cell proliferation *in vitro* and in several animal models. The first clinical study demonstrated the safety and feasibility of local delivery of antisense in the treatment and prevention of restenosis; another randomised clinical trial (AVAIL) with local delivery of c-myc morpholino compound in patients with coronary artery disease demonstrated its long-term effect on reducing neointimal formation, as well as its safety. These preliminary findings from the small cohort of patients require confirmation in a larger trial utilising more sophisticated drug-eluting technologies.

Keywords: angioplasty, antisense oligonucleotides, gene therapy, restenosis, stent

Expert Opin. Biol. Ther. (2005) 5(1):79-89

1. Introduction

Despite significant advances in pharmacological treatment and the implementation of novel surgical techniques, the treatment of coronary artery disease (CAD) is characterised by the expanding use of percutaneous coronary intervention (PCI) [1]. The introduction of stents showed a significant decrease in vessel remodelling and elastic recoil at the site of intervention and clearly demonstrated the superiority of stent implantation over percutaneous transluminal coronary angioplasty (PTCA) alone with respect to restenosis in *de novo* coronary lesions. However, extensive use of coronary stents to prevent restenosis has produced a new disease: in-stent restenosis. Unfortunately, this complication continues to be difficult to prevent; regardless of the treatment strategy, the rate of in-stent restenosis (20 – 60% after bare metal stent implantation) is still unacceptably high, depending on vessel and patient bias [2-4,6]. This is particularly true in patients with diabetes and in some lesion sublets, such as bifurcated lesions, long diffuses lesions, and/or small vessels [6]. However, it is also evident that neointimal proliferation is not affected by the stenting technique [5]. Thus, despite significant advances in the treatment of cardiovascular disease, intimal

10.1517/14712598.5.1.79 ISSN 1471-2598

hyperplasia remains the most common cause of early failure after PCI.

In addition to mechanical procedures, existing treatment strategies for intimal hyperplasia include two main approaches:

- inhibiting vascular smooth muscle cell (VSMC) proliferation and growth, and stimulating the pathways that lead to VSMC apoptosis
- promoting re-endothelialisation and augmenting endothelial functions

Considering the fact that endothelial cells can have heterotopic origin and supply sources, the field of endothelial progenitor cells is being intensely studied at present and might have a high impact for antirestenotic therapies in the future. A new trend towards stent-based drug delivery explored the potential of antiproliferative drugs in the treatment and prevention of the intimal hyperplasia. Although the first clinical experiences with drug-eluting stents have produced stunning results, there are a number of theoretical limitations to these devices, including early thrombosis and late restenosis.

Indeed, several completed studies on sirolimus- and paclitaxel-eluting stents showed great capability of this approach in the prevention and/or treatment of in-stent restenosis. However, recent advances in vascular gene transfer have shown potential new treatment modalities for cardiovascular disease, particularly in the treatment of vascular restenosis.

2. Rationale for using antisense oligonucleotides in the treatment of intimal hyperplasia

2.1 Gene therapy of intimal hyperplasia

Gene therapy, which has been defined as the transfer of nucleic acids (either functional genes or oligonucleotides [ODNs]) to the somatic cells of an individual with a resulting therapeutic effect [7], targets particular genes and thus appears to be more selective and suited to a site-specific treatment approach, as in the case of intimal hyperplasia, than conventional drug therapy. Moreover, vascular gene transfer can be used not only to overexpress or block therapeutically important proteins and correct genetic defects, but also to study various genes and experimentally test their role in the development of particular pathological conditions.

Neointimal hyperplasia involves a complex interaction between multiple growth factors that promotes VSMC migration and proliferation [8-10]. Platelet aggregation and simultaneous activation of smooth muscle cells (SMCs) in the media immediately follow injury to the vessel wall. Within 24 h, DNA replication in the medial SMCs can be observed; in ~ 4 days, migration of SMCs from the media to the intima becomes apparent. In the intima, proliferation of SMCs occurs for several days and stops in ~ 4 weeks, even in the absence of endothelial regeneration. Furthermore, synthesis and deposition of the extravascular matrix leads to intimal hyperplasia [11,12].

Multiple factors have been implicated in the development of intimal hyperplasia. Many of these have been identified and studied. They involve the release of a host of cytokines and growth factors by platelets, leukocytes and SMCs, which can induce the synthesis of gene products that stimulate VSMC migration and proliferation, thereby contributing to excessive intimal growth. These appear to be feasible targets for future therapy and prevention of the intimal hyperplasia. Gene transfer studies (e.g., *eNOS*, *p53*, kallikrein gene, *HSV-tk* with ganciclovir therapy, cytosine deaminase with 5-fluorocytosine therapy, tissue factor pathway inhibitor, adrenomedullin, c-*Myc* antisense) have shown the potential advantages of gene therapy in the prevention of intimal hyperplasia [13-22]. However, several hurdles must be overcome before gene-based stent therapy can be applied successfully in clinical trials. This includes:

- increasing the efficiency of gene delivery through atherosclerotic plaques
- increasing intramural retention times; preventing the inflammatory reaction that stents coated with biodegradable polymers can elicit
- overcoming the risk of systemic gene delivery
- accessing the adventitia via a percutaneous approach [23]

2.2 Antisense approach to inhibiting gene expression

The first successful experience that used ODNs to inhibit gene expression and virus replication was presented by Zamecnik and Stephenson in 1978 [24]. They synthesised a 13-mer oligodeoxynucleotide complementary to the 5′- and 3′-reiterated terminal sequences of the Rous sarcoma virus 35S RNA and showed that exposure of infected fibroblasts to this oligomer led to a 99% decrease in reverse transcriptase activity in the medium, which also correlated with a decrease in cellular transformation. This study showed that such compounds may have a therapeutic advantage by specifically targeting genetic sequences that are critical to disease processes.

Three major classes of ODNs exist:

- antisense sequences (commonly called antisense ODNs)
- antigen sequences
- ribozymes and *cis*-element double-stranded decoy ODNs

Antisense sequences are derivatives of nucleic acids (DNA or RNA sequences) that hybridise cytosolic mRNA strands through hydrogen bonding to complementary nucleic acid bases. Antigene sequences hybridise double-stranded DNA in the nucleus, forming triple helices. Instead of inhibiting protein synthesis simply by binding to a single targeted mRNA, ribozymes combine enzymatic processes with the specificity of base pairing, creating a molecule that can incapacitate multiple targeted mRNAs [25]. Transfection of decoy ODNs will result in attenuation of authentic *cis–trans* interaction, leading to the removal of *trans*-factors from the endogenous *cis*-elements, with subsequent modulation of gene expression [26].

The antisense approach to inhibiting gene expression involves introducing ODNs complementary to mRNA into cells to block any one of the following processes: uncoiling of DNA, transcription of DNA, export of RNA, DNA splicing, RNA stability, or RNA translation involved in the synthesis of proteins in cellular proliferation [27]. It includes the use of antisense ODNs, antisense mRNA, autocatalytic ribozymes and the insertion of a section of DNA to form a triple helix. The inhibition of gene expression thus achieved is believed to be highly specific and is dependent on formation of the antiparallel duplex by complementary base pairing between the antisense DNA and the target mRNA, in which adenosine and thymidine or guanosine and cytidine interact through hydrogen bonding. This elegant specificity of the Watson–Crick base pairing between the ODN and the target mRNA may form the basis for a highly effective and specific therapeutic modality and might be used to eliminate the expression of any cellular protein [28].

ODNs that are complementary or antisense to individual mRNA sequences bind to the particular sequence and prevent translation [29]. Once inside the cell, the ODN binds to its target mRNA in the cytoplasm, nucleus, or both. This hybridisation with the mRNA explains two main mechanisms of action of the ODNs [30,31]. First, it has been suggested that ODNs exert steric interference [32] to ribosome binding and translation, or splice excision. Evidence for steric interference came from studies in which antisense to the 5′ cap of mRNA was found to be most effective in inhibiting rabbit β-globin protein synthesis [33]; the 5′ cap is the site where a number of initiation factors bind for ribosome assembly, the unwinding of DNA, and ribosome translocation along the mRNA [34].

Second, the effect of antisense ODNs is due to the induction of cleavage of mRNA by the nuclease RNAse H that specifically recognises DNA–RNA duplexes [35-37]. Antisense ODNs can also enter the nucleus where they may inhibit splicing [38], preventing the process of pre-mRNA or mRNA, or block transport of the mRNA out of the nucleus. Introduction of ODNs thus results in a reduction of specific mRNA and protein levels if mediated by RNAse H, or a reduction in specific protein levels if mediated by steric interference.

It was also found that SMC proliferation could be inhibited by antisense oligomers via non-antisense mechanisms [39]. In this case, the presence of four contiguous guanosine residues (G-4 tract) within the ODN sequence caused a sequence-specific, but not antisense-dependent, antiproliferative effect.

2.3 Efficacy of antisense oligonucleotides in the prevention of restenosis

Inhibition of several cellular proto-oncogenes, including the DNA binding protein c-*Myb* [40,41], non-muscle myosin heavy chain, proliferating cell nuclear antigen (PCNA) [42,43], and also platelet-derived growth factor (PDGF) [44], basic fibroblast growth factor (bFGF) [45], c-*raf* [46] and c-*Myc* [47,48], has been shown to inhibit SMC proliferation *in vitro*; the efficacy of these oligomers has also been confined in *in vivo* studies [49-51]. Most recent data has shown great efficacy of antisense ODNs in a study of fibroblast growth factor–receptor interaction and has revealed possible new sites targeted for restenosis prevention [52]. Another study found *in vivo* that downregulation of N-cadherin expression by antisense transfection significantly altered cell-cell adhesion, decreased SMC migration and prevented restenosis [53]. These *in vitro* and *in vivo* studies not only demonstrated efficacy of the ODN in the inhibition of SMC migration, proliferation and intimal hyperplasia, but also revealed key points in the development of antisense therapy of arterial restenosis (**Table 1**).

The combination of two different ODNs has demonstrated an inhibitory effect on arterial intimal hyperplasia following balloon injury [54]. Even after single intraluminal delivery, the antisense oligomer combination directed against PCNA and cell division cycle 2 kinase (cdc2) was effective in suppressing neointima formation in the rat model of carotid artery balloon injury [50]. Another combination of ODNs, antisense cdc2 and cdk2 ODNs was successfully used by Abe *et al.* [55] to suppress neointimal SMC accumulation *in vivo* in the rat carotid artery. At the same time, Robinson *et al.* [56] demonstrated that single endoluminal delivery of PCNA/cdc2 antisense ODNs by porous balloon catheter does not affect neointima formation or vessel size in the pig coronary artery model of post angioplasty restenosis.

The time frame of antisense ODN introduction to injured vessel may play an important role in the prevention of restenosis. Schmidt *et al.* [57] showed that rat carotid artery SMC proliferation begins 1 – 2 days after balloon catheter-induced injury, and entry of cells into the growth phase was completed within 3 days of injury. At the same time, minimally modified bFGF-specific antisense ODN exerted its antiproliferative activity within this time frame. This strategy has also been successfully applied in the inhibition of various targets, such as c-cbl and c-src [58], HSV-1 [59], and c-Myc [47].

Different structural types of antisense ODN demonstrate different efficacy and specificity in inhibiting targeted mRNA. Stein *et al.* [60] carried out cell-free translation studies to compare the efficacy and specificity of four antisense structural types: DNA, phosphorothioate DNA, 2'-O-methyl RNA, and morpholino ODNs, a novel antisense ODN. It was shown that at low concentrations of antisense oligomer, all four types provide high specificity, but the morpholino oligos and 2'-O-methyl RNA afford better efficacy. At high oligomer concentrations, all four types provide high efficacy, although the morpholino oligos and 2'-O-methyl RNA provide substantially better specificity than the DNA and S-DNA. It was also shown that mRNA could discriminate between ODNs that differ only by one or two bases [30,60,61]. Changes in a c-Myc antisense ODN sequence of only two bases resulted in an almost complete loss of its activity [61].

Frequent nonspecific effects may follow the use of antisense ODNs. Some of these effects are sequence-specific, as described for 4-guanosine residue, which causes an aptamer

Table 1. Experimental data on antisense therapy of intimal hyperplasia.

Investigators	Experimental model	Gene	Delivery vehicle
Simons *et al.*	Rat	c-myb	Pluronic gel
Edelman *et al.*	Rat	c-myb	EVac
Azrin *et al.*	Pig	c-myb	Hydrogel catheter
Gunn *et al.*	Pig	c-myb	None
Bennet *et al.*	Rat	c-myb	Pluronic gel
Edelman *et al.*	Rat	c-myb	EVac
Shi *et al.*	Pig	c-myb	None
Morishita *et al.*	Rat	cdc-2/PCNA	HVJ
Abe *et al.*	Rat	cdc-2	Pluronic gel
Abe *et al.*	Rat	cdk-2	Pluronic gel
Morishita *et al.*	Rat	cdk-2	HVJ
Simons *et al.*	Rat	PCNA	Pluronic gel
Siroios *et al.*	Rat	PDGF-α receptor	EVac
Robinson *et al.*	Pig coronary artery	PCNA/cdc kinase	Local delivery catheter
Biro *et al.*	Rat carotid	c-myc	None
Kipshidze *et al.*	Rabbit femoral artery	c-myc	Transport catheter
Kipshidze *et al.*	Pig coronary artery	c-myc	Phosphorylcholine-coated stent

EVac: Ethylenevinylacetate; HVJ: Haemagglutinating virus of Japan; NR: Not reported; PCNA: Proliferating cell nuclear antigen; PDGF: Platelet-derived growth factor.

effect, leading to non-antisense-dependent inhibition [39]. Although *in vitro* studies have clearly established that antisense oligomers can inhibit target genes without producing gross toxic effects on cultured cells, *in vivo* studies in *Xenopus* oocytes reveal that it is not possible to obtain specific cleavage of an intended target RNA without also causing at least the partial destruction of many non-targeted RNA [42].

2.4 Limitations of antisense therapy

Despite the apparent success of antisense ODN therapy, several limitations of this technology have manifested. First, the ODN must effectively cross the cell membrane to reach the cytoplasm or nucleus (permeation). Once inside the cell, the ODN must be resistant to degradation (stability). Finally, the ODN must be able to bind specifically and with a high affinity to the RNA target in order to inhibit the desired gene (affinity and specificity) [28,30].

ODNs are strongly negatively charged, which prevents them from passing the cell surface passively. Uptake of ODNs appears to occur by receptor-mediated endocytosis and is determined by various factors, including the length of the ODN, the total charge of the molecule, its lipid solubility and the nucleotide concentration [62,63].

Naturally occurring nucleotide oligomers are easily and rapidly degraded by exo- and endonucleases, which can significantly limit their utilisation in antisense technology [64,65]. Previous studies have confirmed that the presence of simple 3′ or 3′ plus 5′ modifications may provide protection from degradation by exonucleases [66,67]. However, the action of intracellular endonucleases is sufficient to degrade the end-modified oligomers; uniform modification throughout the oligomers has been suggested. Interestingly, previous studies [41] have shown that unmodified ODNs are more efficacious *in vivo* and *in vitro* than modified ODNs.

The affinity of the ODNs depends on their length and base composition. An increase in ODN length also increases its affinity; however, after a particular ODN length has been reached, its affinity decreases. The affinity also increases as the number of guanosine-cytidine pairs increase [68]. The effect of ODNs is believed to be highly specific due to complementary base pairing between the antisense DNA and the target mRNA, but it does not prevent the frequent nonspecific effects described earlier [42].

3. Clinical implications and first experience of antisense therapy in the treatment of vascular proliferative disease

3.1 Delivery systems for antisense oligonucleotides

One of the most important technical problems in the clinical applicability of antisense technology is the development of an efficient and suited delivery system for ODNs. Local drug delivery was designed to bring the antisense agent to the coronary artery during the period of time corresponding to peak-injury response. The earliest attempts to deliver antisense agents to prevent restenosis involved a rat carotid artery model using adventitial [49] or surgical application [50]. The initial clinically applicable devices were catheter-based and provided local delivery as a bolus injection, at which time the catheter

was withdrawn. The combination of antisense targeting to c-Myc with catheter-based delivery to coronary arteries of pigs for the prevention of restenosis began with phosphorothioate ODNs [29]. The bolus injection of phosphorothioate oligomers produced a reduction in heart rate, blood pressure and cardiac output in primate models that was sometimes lethal [69-72].

Modified angioplasty balloons have been designed and developed for local delivery of genes or drugs into the vascular wall [73]. Some examples of modified angioplasty balloons are the double balloon catheter, in which the agent is infused into a closed compartment between the balloons and can diffuse with minimal pressure onto the vessel wall; perforated balloon, in which the agent is infused under pressure through pores in the balloon wall and onto the vessel tissue; and the hydrogel-coated balloon, in which the agent is mixed in hydrogel, which dissolves in the bloodstream when the balloon is inflated and pressed against the vessel wall (the agent can then diffuse into the luminal cells). The limitation of these devices is pressure-driven delivery that causes additional vessel damage and low efficacy. Viral vectors or different lipid carriers may increase efficacy of delivery. Fibrin meshwork is an alternative vehicle for sustained release of antisense, a factor that may be important in the case of stent implantation.

Polymer-coated stents have been used successfully to deliver micromolar concentrations of c-Myc antisense phosphorodiamidate morpholino oligomers (PMOs) into the vessel wall [74]. Zhang *et al.* [75] reported effective local delivery of c-Myc antisense ODN by gelatin-coated platinum-ipidium stents in rabbits. This experience showed that ultimate success will require polymers that are capable of rapid elution of the ODN with minimal capacity to inflame or otherwise cause additional injury to the vessel wall.

Perfluorobutane gas microbubbles with a coating of dextrose and albumin efficiently bind antisense oligomers [76]. These 0.3 – 10 μm particles bind to sites of vascular injury. Furthermore, perfluorobutane gas is an effective cell membrane fluidiser. The potential advantages of microbubble carrier delivery include minimal additional vessel injury from delivery; no resident polymer to degrade, leading to eventual inflammation; rapid bolus delivery; and the high likelihood of repeated delivery. In addition, the potential for PGMC to deliver to vessel regions both proximal and distal to stents in vessels suggests that this mode of delivery will serve as an excellent adjuvant to a variety of catheter and coated-stent delivery techniques.

3.2 First clinical experience of antisense therapy in the treatment of restenosis

The clinical applicability of antisense technology remains limited by a relative lack of specificity, slow uptake across the cell membrane, and rapid degradation of ODNs.

Promising results emerged from the Proliferation Reduction with Vascular Energy Trial (PREVENT) trial [77], which showed efficacy of *ex vivo* gene therapy of human vascular bypass grafts with a decoy ODN to the E2F transcription factor, which is essential for VSMC proliferation in lowering the incidence of venous bypass graft failure. Recently reported results of another clinical trial (ITALICS [randomized Investigation by the Thoraxcenter of Antisense DNA using Local delivery and Ivus after Coronary Stenting]) in Rotterdam [78] that examined the effectiveness of an antisense compound directed against c-Myc, however, were disappointing (Table 2). The authors considered several reasons for the observed lack of effect of the antisense compound. Among them, the local concentration of antisense compound achieved may not have been high enough to show a significant effect. Furthermore, the single administration of the antisense compound might not be effective in suppressive c-Myc, which showed biphasic response to the vessel injury. The authors also used a self-expanding stent, which can cause chronic injury of stented arteries. Under these circumstances, a single injection of antisense may not be adequate to reduce myointimal response.

Table 2. Results of ITALICS trial that examined the effectiveness of antisense directed against c-Myc, showing a lack of effect of the antisense compound in comparison with placebo.

Results	Placebo n = 39	Antisense n = 38	P value
Loss index	0.66 ± 0.3	0.71 ± 0.3	0.5
Restenosis rate	38%	34%	0.8

ITALICS: Randomized Investigation by the Thoraxcenter of Antisense DNA using Local delivery and Ivus after Coronary Stenting.

Optimistic results have been obtained with the newly introduced AVI-4126, which belongs to a family of molecules known as the PMOs [28]. These oligomers are comprised of (dimethylamino) phosphinylideneoxy-linked morpholino subunits, which contain a heterocyclic base recognition moiety of DNA attached to a substituted morpholine ring system. In general, PMOs are capable of binding to RNA in a sequence-specific fashion with sufficient avidity to be useful for the inhibition of the translation of mRNA into protein *in vivo*.

Although PMOs share many similarities with other substances that are capable of producing antisense effects (e.g., DNA, RNA and their ODN analogues, such as the phosphorothioates [PSOs]), there are several critical differences. Most importantly, PMOs are uncharged and resistant to degradation under biological conditions, exceptionally stable at temperature extremes, and resistant to degradation in plasma and to the nucleases found in serum and liver extracts [79]. They also exhibit a high degree of specificity and efficacy, both *in vitro* and in cell culture [80], which averts a variety of potentially significant limitations observed in PSO chemistry. The antisense mechanism of action appears to be through the PMO hybrid duplex with mRNA to inhibit translation. Finally, PMOs have demonstrated antisense activity against c-Myc pre-mRNA in living human cells [81]. The combined efficacy, potency and lack of nonspecific activities of PMO chemistry have compelled us to re-examine the approach to antisense to c-Myc in the prevention of restenosis following balloon angioplasty.

Table 3. Study with endoluminal delivery of advanced c-Myc antisense PMO (Resten-NG) into the area of PCI demonstrated complete inhibition of c-Myc expression and a significant reduction of the neointimal formation in the treated vessels in a dose-dependent fashion while allowing for complete vascular healing.

	Control (n = 6)	1 mg (n = 8)	5 mg (n = 9)	10 mg (n = 7)
Injury Score	0.95	0.91	0.90	0.94
Lumen	3.26	4.76	4.91	5.62
Media	2.05	1.97	2.04	2.16
Intima	3.88	2.81	2.01	1.95
IA/IS	4.08	3.09	2.17	2.13

IA: Intima area; IS: Injury score; PMO: Phosphorodiamidate morpholino oligomer; PCI: Percutaneous coronary intervention.

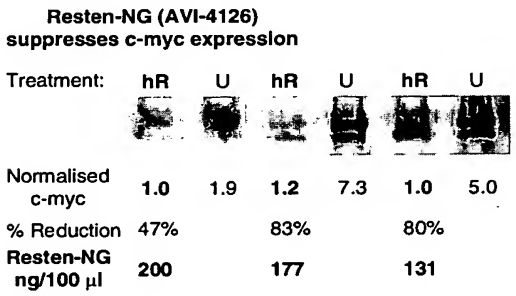

Figure 1. Impact of the advanced c-Myc antisense PMO (Resten-NG; AVI-4126)-eluting phosphorylcholine-coated stents (DES) on c-myc expression.
DES: Drug-eluting stents; PMO: Phosphorodiamidate morpholino oligomer.

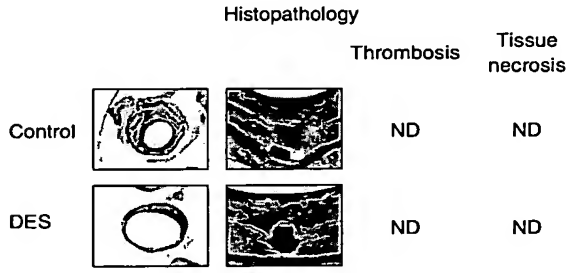

Figure 2. Impact of the advanced c-Myc antisense PMO (Resten-NG)-eluting phosphorylcholine-coated stents (DES) on the vessel wall.
DES: Drug-eluting stents; PMO: Phosphorodiamidate morpholino oligomer.

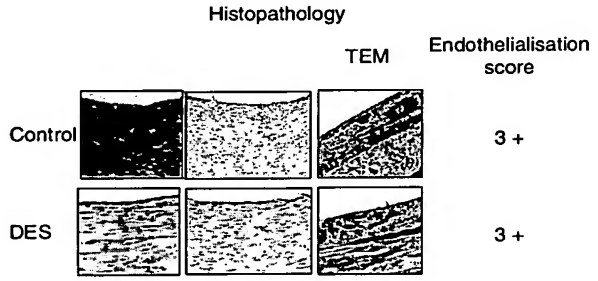

Figure 3. Impact of the advanced c-Myc antisense PMO (Resten-NG; AVI-4126)-eluting phosphorylcholine-coated stents (DES) on the vessel wall.
DES: Drug-eluting stents; PMO: Phosphorodiamidate morpholino oligomer; TEM: Transmission electron microscopy.

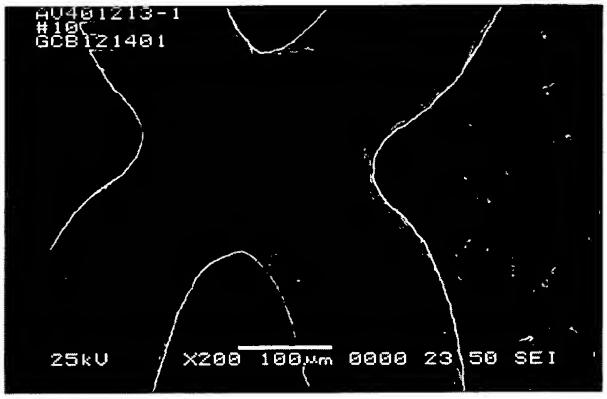

Figure 4. SEM of antisense-eluting polymer-coated stent. Courtesy of Medtronic, Inc.
SEM: Scanning electron microscopy.

PMOs have been evaluated for adverse effects after intravenous bolus injections in both primates (Good Laboratory Practice studies by Sierra Biomedical) and humans (Good Clinical Practice studies at MDS Harris). No alterations in heart rate, blood pressure or cardiac output were observed. In summary, bolus injections of PMO by local catheter-based delivery devices are feasible.

The authors' studies with endoluminal delivery of advanced c-Myc antisense PMO (Resten-NG; AVI-4126 for restenosis) into the area of PTCA (Transport Catheter™; rabbit iliac artery model) [82] and into coronary arteries following stent implantation (Infiltrator™ delivery system; pig model) [83] (**Table 3**) demonstrated complete inhibition of c-Myc expression and a significant reduction of the neointimal formation in the treated vessels in a dose-dependent

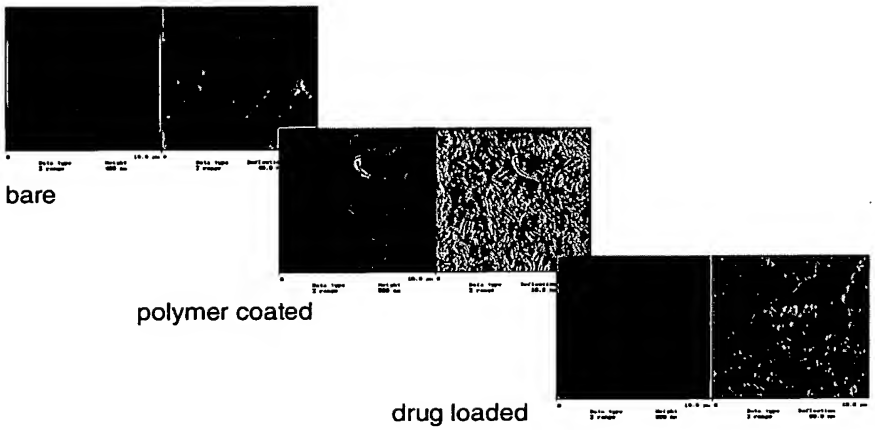

Figure 5. Antisense-eluting stent. Surface of the metallic, polymer-coated, and AVI-4126-eluting polymer-coated stents viewed using atomic force microscopy. Courtesy of Medtronic, Inc.

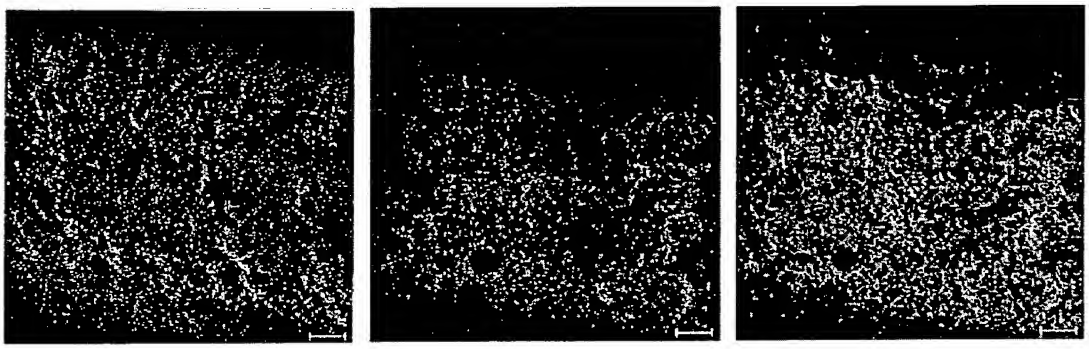

Figure 6. Antisense-eluting stent. Surface analysis techniques – imaging SIMS. Homogenous distribution of AVI-4126 on polymer-coated stents. Courtesy of Medtronic, Inc.
SIMS: Secondary ion mass spectroscopy.

fashion while allowing for complete vascular healing. Similar results were obtained after implantation of advanced c-Myc antisense PMO-eluting phosphorylcholine-coated stents in the porcine coronary restenosis model [74] (**Figures 1 – 3**). Less inflammation was also observed after implantation of the antisense-loaded stent. This favourable influence on hyperplasia (a 40% reduction of intima) in the absence of endothelial toxicity may represent an advantage of antisense PMO over more destructive methods, such as brachytherapy [84] or cytotoxic inhibitors [85]. The authors believe that local application of antisense via a polymer-coated stent may be more preferable then local catheter delivery (**Figures 4 – 6**). The authors also tested novel perfluorocarbon gas microbubble carriers for site-specific delivery of AVI-4126 to the injured vessel wall and obtained encouraging results [86].

The most robust of observations to date by multiple investigators is the finding that AVI-4126 is safe and effective in vascular application in a number of species. Different methods for local delivery have also been tested, but these observations fall short of proof that AVI-4126 will be effective in the treatment of human restenosis. Efficacy in animal models has also been encouraging. Furthermore, all these studies with AVI-4126 indicated that the agent is safe.

The last remaining question is if AVI-4126 will find a place in future therapeutic regimens for the prevention of restenosis; this answer might be found in the results of Phase II clinical studies being conducted at present, such as AVAIL (safety and efficacy of AVI-4126 Antisense delivered via Infiltrator catheter Locally after PCI in humans). Recent data from a 6-month follow-up on the patients enrolled in the

Table 4. AVAIL study showed that antisense can be as effective in the prevention of restenosis as most well-known antiproliferative agents.

	Control	3 mg	10 mg
Ref diameter (mm)	2.63 ± 0.40	2.79 ± 0.78	2.77 ± 0.46
MLD pre	0.65 ± 0.40	0.94 ± 0.71	1.10 ± 0.50
MLD post	2.81 ± 0.98	3.00 ± 0.63	2.84 ± 0.71
MLD @ FU	1.55 ± 0.64	1.69 ± 0.99	2.00 ± 0.74
% DS:			
pre	71.10 ± 18.04	68.35 ± 19.80	60.58 ± 15.50
Post	6.85 ± 8.94	8.82 ± 7.49	9.71 ± 9.58
FU	39.45 ± 22.03	41.91 ± 24.86	22.23 ± 21.23
Late loss	1.26 ± 0.19	1.45 ± 0.19	0.74 ± 0.16*
Binary restenosis			
Number	3/9	4/12	1/12
%	33.3	33.3	8.3

*ANOVA significant difference, $p < 0.03$.
ANOVA: Analysis of variance; AVAIL: Safety and efficacy of AVI-4126 Antisense delivered via Infiltrator catheter Locally after PCI in humans; DS: Diameter stenosis; FU: Follow-up; MLD: Minimal luminal diameter; PCI: Percutaneous coronary intervention.

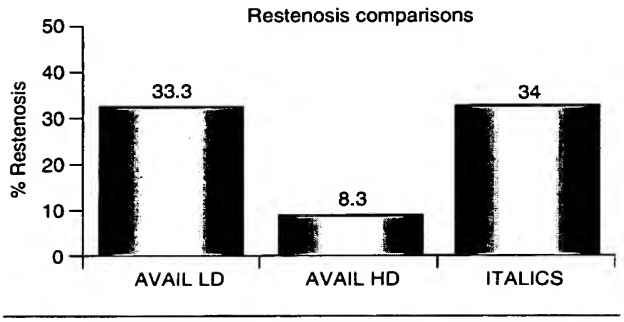

Figure 7. AVAIL study showed that antisense can be as effective in the prevention of restenosis as most well-known antiproliferative agents.
AVAIL: Safety and efficacy of AVI-4126 Antisense delivered via Infiltrator catheter Locally after PCI in humans; HD: High dose; ITALICS: Randomized Investigation by the Thoraxcenter of Antisense DNA using Local delivery and Ivus after Coronary Stenting; LD: Low dose; PCI: Percutaneous coronary intervention.

AVAIL study [87] showed that AVI-4126 is effective in reducing neointimal formation, particularly when locally delivered at a high dose. It was also concluded that local delivery of antisense is safe and feasible. The results (Table 4 and Figure 7) indicate that antisense (AVI-4126) can be as effective in the prevention of restenosis as most well-known antiproliferative agents, but in contrast with other chemotherapeutics (paclitaxel, actinomycin D), c-Myc antisense inhibits the cell cycle in the G-1 phase, which make its effect less toxic and comparable to that of rapamycin.

4. Expert opinion and conclusion

Proof of principle has been established that inhibition of several cellular proto-oncogenes, including the DNA binding protein c-Myb, non-muscle myosin heavy chain, PCNA, PDGF, bFGF and c-Myc, inhibit SMC proliferation *in vitro* and in animal models. The first clinical study demonstrated the safety and feasibility of local delivery of antisense in the treatment and prevention of restenosis; another randomised clinical trial (AVAIL) with local delivery of c-Myc morpholino compound in patients with CAD demonstrated its long-term effect on reducing neointimal formation, as well as its safety. These preliminary findings from the small cohort of patients require confirmation in a larger trial utilising more sophisticated drug-eluting technologies.

Further identification of new transcriptional factors and signalling mediators would be an important step in the development of new potential targets for therapy of vascular restenosis.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. SIMONSEN M: Changing role for cardiac surgery as use of stents continues growth. *Cardiovasc. Device Update* (2003) 9:1-7.
2. TOPOL EJ, SERRUYS PW: Frontiers in interventional cardiology. *Circulation* (1998) 98:1802-1820.
3. SERRUYS PW, FOLEY DP, SUTTORP MJ *et al.*: A randomized comparison of the value of additional stenting after optimal balloon angioplasty for long coronary lesions. *J. Am. Coll. Cardiol.* (2002) 39:393-399.
4. VAN DEN BRAND MJ, RENSING BJ, MOREL MA *et al.*: The effect of completeness of revascularization on event-free survival at one-year in the ARTS trial. *J. Am. Coll. Cardiol.* (2002) 39:559-564.

5. NAKATANI M, TAKEYAMA Y, SHIBATA M *et al.*: Mechanisms of restenosis after coronary intervention. Difference between plain old balloon angioplasty and stenting. *Cardiovasc. Pathol.* (2003) 12:40-48.
6. GOLDBERG SL, LOUSSARARIAN A, DE GREGORIO J, DI MARIO C, ALBIERRO R, COLOMBO A: Predictors of diffuse and aggressive intrastent restenosis. *J. Am. Coll. Cardiol.* (2001) 37:1019-1025.
7. YLA-HERTTUALA S, MARTIN JF: Cardiovascular gene therapy. *Lancet* (2000) 355:213-222.
8. LIBBY P, SCHWARTZ D, BOGI E, TANAKA H, CLINTON SK: A cascade model for restenosis: special case of atherosclerosis progression. *Circulation* (1992) 86:47-52.
9. CLOWES AW, CLOWES MM, FINGERLE J, REIDLY MA: Regulation of smooth muscle cell growth in injured artery. *J. Cardiovasc. Pharmacol.* (1989) 14(Suppl. 6):S12-S15.
10. FINGERLE J, JOHNSON R, CLOWES AW, MAJESKY MW, REIDY MA: Roles of platelets in smooth muscle cell proliferation and migration after vascular injury in rat carotid artery. *Proc. Natl. Acad. Sci. USA* (1989) 86:8412-8416.
11. NIKKARI ST, CLOWES AW: Restenosis after vascular reconstruction. *Ann. Med.* (1994) 26:95-100.
12. SCHWARTZ SM, DE BLOIS D, O'BRIEN RM: The intima – soil for restenosis and atherosclerosis. *Circ. Res.* (1997) 77:445-465.
13. AGATA J, ZHANG JJ, CHAO L: Adrenomedullin gene delivery inhibits neointima formation in rat artery after ballon angioplasty. *Regul. Rep.* (2003) 112:115-120.
14. KIPSHIDZE N, MOSES J, SHANKAR LR *et al.*: Perspectives on antisense therapy for the prevention of restenosis. *Curr. Opin. Mol. Ther.* (2001) 3:265-277.
15. KIPSHIDZE N, IVERSEN P, KEANE E *et al.*: Complete vascular healing and sustained suppression of neointimal thickening after local delivery of advanced c-*myc* antisense at six months follow-up in a rabbit balloon injury model. *Cardiovasc. Radiat. Med.* (2002) 3:26-30.
16. GEORGE SJ, ANDELINI GD, CAPOGROSSI MC *et al.*: Wild-type p53 gene transfer inhibits neointima formation in human saphenous vein by modulation of smooth muscle cell migration and induction of apoptosis. *Gene Ther.* (2001) 8:668-676.
17. MURAKAMI H, YAYAMA K, MIAO RQ *et al.*: Kallikrein gene delivery inhibits vascular smooth muscle cell growth and neointima formation in the rat artery after balloon angioplasty. *Hypertension* (1999) 34:164-170.
18. STEG GP, TAHLIL O, AUBAILLY N *et al.*: Reduction of restenosis after angioplasty in an atheromatous rabbit model by suicide gene therapy. *Circulation* (1997) 96:408-411.
19. HARELL RL, RAJANAYAGAM S, DOANES AM *et al.*: Inhibition of vascular smooth muscle cell proliferation and neointimal accumulation by adenovirus-mediated gene transfer of cytosine deaminase. *Circulation* (1997) 96:621-627.
20. ZOLDHELIY P, MCNATT J, SHELAT H *et al.*: Thromboresistence of balloon-injured porcine carotid arteries after local gene transfer of human tissue factor pathway inhibitor. *Circulation* (2000) 101:289-295.
21. VAN BELLE E, TIO FO, CHEN D *et al.*: Passivation of metallic stents after arterial gene transfer of phVEGF 165 inhibits thrombus formation and intimal thickening. *J. Am. Coll. Cardiol.* (1997) 29:1371-1379.
22. YOON J, WU CJ, HOMME J *et al.*: Local delivery of nitric oxide from an eluting stent to inhibit neointimal thickening in a porcine coronary injury model. *Yonsei Med. J.* (2002) 43:242-251.
23. FELDMAN MD, BO SUN, KOCI B *et al.*: Stent-based gene therapy. *J. Long Term Eff. Med. Implants* (2000) 10:47-68.
24. ZAMECNIK P, STEPHENSON M: Inhibition of Rous sarcoma virus replication and cell transformation by a specific deoxyoligonucleotide. *Proc. Natl. Acad. Sci. USA* (1978) 75:280-284.
•• **Describes the first attempt to inhibit gene expression using antisense ODNs.**
25. WANG A, CREASY A, LARDNER M *et al.*: Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science* (1985) 228:149-154.
26. MORISHITA R, KANEDA Y, OGIHARA T: Therapeutic potential of oligonucleotide-based therapy in cardiovascular disease. *BioDrugs* (2003) 17(6):383-389.
27. HELENE C, TOULME JJ: Specific regulation of gene expression by antisense, sense and antigene nucleic acids. *Biochem. Biophys. Acta* (1990) 1049:99-125.
28. STEIN CA, CHENG YC: Antisense oligonucleotides as therapeutic agents - is the bullet really magical? *Science* (1993) 261:1004-1012.
29. SHI Y, FAD A, GALLEON A *et al.*: Transcatheter delivery of c-myc antisense oligomers reduced neointimal formation in a porcine model of coronary artery balloon injury. *Circulation* (1994) 90:944-951.
•• **One of the first papers to discuss the efficacy of local c-myc antisense delivery to prevent intimal hyperplasia *in vivo*.**
30. BENNETT MR, SCHWARTZ SM: Antisense therapy for angioplasty restenosis: some critical considerations. *Circulation* (1995) 92:1981-1993.
31. STEIN CA, TOKINSON JL, YAKUBOV L: Phosphorothioate oligodeoxynucleotides antisense inhibitors of gene expression? *Pharmacol. Ther.* (1991) 52:365-384.
32. BOLZIAU C, KURFIST R, CAZENAVE C, ROIG V, THOUNG NT, TOULME JJ: Inhibition of translation initiation by antisense oligonucleotides via an RNAase independent mechanism. *Nucleic Acid Res.* (1991) 19:1113-1119.
• **Describes possible mechanism of the antisense inhibition through steric interference.**
33. GOODCHILD J: Inhibition of gene expression by oligonucleotides. In: *Oligonucleotides: Antisense Inhibitors of Gene Expression.* Cohen J (Ed.), Macmillan Press, London, UK (1989):53-77.
34. KOZAK M: Influences of mRNA secondary structure on inhibition by eucaryotic ribosome. *Proc. Natl. Acad. Sci. USA* (1996) 83:2850-2854.
35. WAGNER R, NISHIKURA K: Cell cycle expression of RNA duplex unwinding activity in cells. *Mol. Cell. Biol.* (1988) 8:770-777.
36. DASH P, LOTAN L, KNAPP M, KANDEL ER, GOELET P: Selective elimination of mRNA *in vivo*: complementary oligodeoxynucleotides promote RNA degradation by RNAse-H like activity. *Proc. Natl. Acad. Sci. USA* (1987) 84:7896-7900.

37. DAGLE JM, WALDER JA, WEEKS DL: Target degradation of mRNA in *Xenopus* oocytes and embryos directed by modified oligonucleotides: studies of An2 and cyclin in embryogenesis. *Nucleic Acid Res.* (1990) 18:4751-4757.

38. MCMANNAWAY ME, NECKERS LM, LOKE SL *et al.*: Tumor-specific inhibition of lymphoma growth by an antisense oligodeoxynucleotide. *Lancet* (1990) 335:808-811.

39. BURGESS TL, FISHER EF, ROSS SL *et al.*: The antiproliferative effect of c-myb and c-myc antisense oligonucleotides in smooth muscle cells is caused by a non antisense mechanism. *Proc. Natl. Acad. Sci. USA* (1995) 92(9):4051-4055.

40. SIMONS M, ROSENBURG RD: Antisense non-muscle, myosin, heavy chain and c-myb oligonucleotides suppress smooth muscle cell proliferation *in vitro*. *Circ. Res.* (1992) 70:835-843.

41. GUNN J, HOLT CM, FRANCIS SE *et al.*: The effect of oligonucleotides to c-myb on vascular smooth muscle cell proliferation and neointima formation after porcine coronary angioplasty. *Circ. Res.* (1997) 80:520-531.

42. SPEIR E, EPSTEIN SE: Inhibition of smooth muscle cell proliferation by an antisense deoxyoligonucleotide targeting the mRNA coding proliferating cell nuclear antigen. *Circulation* (1992) 86:538-547.

43. SIMONS M, EDELMAN ER, ROSENBERG RD: Antisense PCNA oligonucleotides inhibit neointimal hyperplasia in a rat carotid artery injury model. *J. Clin. Invest.* (1994) 93:2351-2356.

44. SUGIKI H: Suppression of vascular smooth muscle cell proliferation by an antisense oligonucleotide against PDGF receptor. *Hokkaido Igaku Zasshi* (1995) 70(3):485-495.

45. HANNA AK, FOX JC, NECKLIS DG *et al.*: Antisense basic fibroblast growth factor gene transfer reduces neointimal thickening after arterial injury. *J. Vasc. Surg.* (1997) 25(2):320-325.

46. MANDIYAN S, SCHUMACHER C, CIOFFI C *et al.*: Molecular and cellular characterization of baboon C-Raf as target for antiproliferative effects of antisense oligonucleotides. *Antisense Nucleic Acid Drug Dev.* (1997) 7(6):539-548.

47. BIRO S, FU YM, YU ZX, EPSTEIN SE: Inhibitory effects of oligodeoxynucleotides targeting c-myc RNA on smooth muscle cell proliferation and migration. *Proc. Natl. Acad. Sci. USA* (1993) 90:654-658.

48. DAUM T, ENGELS JW, MAG M *et al.*: Antisense deoxynucleotide: inhibitor of splicing of mRNA of human immunodeficiency virus. *Int. Virology* (1992) 89:7031-7035.

49. SIMONS M, EDELMAN ER, DEKEYSER J-L, LANGER R, ROSENBERG RD: Antisense c-myb oligonucleotides inhibits intimal arterial smooth muscle cell accumulation *in vivo*. *Nature* (1992) 359:67-70.

50. MORISHITA R, GIBBONS GH, ELLISON KE *et al.*: Single intraluminal delivery of antisense cdc kinase PCNA results in chronic inhibition of neointimal hyperplasia. *Proc. Natl. Acad. Sci. USA* (1993) 90:8474-8478.

51. BAYEVER E, IVERSEN PL, BISHOP MR *et al.*: Systemic administration of a phosphorothioate oligonucleotide with a sequence complementary to p53 for acute myelogenous leukemia and myelodysplastic syndrome: initial results of a Phase I trial. *Antisense Res. Dev.* (1993) 4(4):383-390.

52. AGROTIS A, KANELLAKIS P, KOSTOLIAS G *et al.*: Proliferation of neointimal smooth muscle cells after arterial injury: dependency on interaction between fibroblast growth factor receptor-2 and fibroblast growth factor-9. *J. Biol. Chem.* (2004) 279(40):42221-42229.

53. BLINDT R, BOSSERHOFF AK, DAMMERS J *et al.*: Downregulation of N-cadherin in the neointima stimulates migration of smooth muscle cells by RhoA deactivation. *Cardiovasc. Res.* (2004) 62(1):212-222.

54. SUMMERTON J, STEIN D, HUANG B, MATTHEWS P, WELLER D, PARTRIDGE M: Morpholino and phosphorothioate antisense oligomers compared in cell-free and in-cell systems. *Antisense Nucleic Acid Drug Dev.* (1997) 7:63-70.

55. ABE J, ZHOU W, TAGUCHI J: Suppression of neointimal smooth muscle cell accumulation *in vivo* by antisense cdc2 and cdk2 oligonucleotides in rat carotid artery. *Biochem. Biophys. Commun.* (1994) 198:16-24.

56. ROBINSON KA, CHRONOS NA, SCHIEFFER E *et al.*: Endoluminal local delivery of PCNA/cdc2 antisense oligonucleotides by porous balloon catheter does not affect neointima formation or vessel size in the pig coronary artery model of post angioplasty restenosis. *Cathet. Cardiovasc. Diagn.* (1997) 41:348-353.

57. SCHMIDT A, SINDERMANN J, PEYMAN A *et al.*: Sequence specific antiproliferative effects of antisense and end-capping modified antisense oligodeoxynucleotides targeted against the 5'-terminus of Basic-fibroblast growth factor mRNA in coronary smooth muscle cells. *Eur. J. Biochem.* (1997) 248(2):543-549.

58. TANAKA S, AMLING M, NEFF L *et al.*: c-cbl downstream of c-src in a signaling pathway necessary for bone resorption. *Nature* (1996) 383:528-531.

59. PEYMAN A, HELSBERG M, KRETZSCHMAR G, MAG M, RYTE A, UHLMANN E: Nuclease stability as dominant factor in the antiviral activity of oligonucleotides directed again HSV-1 IEI10. *Antiviral Res.* (1997) 33:135-139.

60. STEIN D, FOSTER E, HUANG SB, WELLER D, SUMMERTON J: A specificity comparison of four antisense types: morpholino, 2'-O methyl RNA, DNA and phosphorothioate DNA. *Antisense Nucleic Acid Drug Dev.* (1997) 7:151-157.

61. HOLT JT, RENDER RL, NELHUS AW: An oligomer complementary to c-myc RNA inhibits proliferation of HL-60 promyelocytic cells and induces differentiation. *Mol. Cell. Biol.* (1988) 8:963-973.

62. VILLA AE, GUZMAN LA, POPTIC EJ *et al.*: Effects of antisense c-myb oligonucleotides on vascular smooth muscle cell proliferation and response to vessel wall injury. *Circ. Res.* (1995) 76:505-513.

63. MULLER DW: The role of proto-oncogenes in coronary restenosis. *Prog. Cardiovasc. Dis.* (1997) 40(2):117-128.

64. WICKSTROM E: Antisense c-myc inhibition of lymphoma growth. *Antisense Nucleic Acid Drug Dev.* (1997) 7(3):225-228.

65. CAZENAVE C, LOREAU N, THUONG NT, TOULME JJ: Enzymatic amplification of translation inhibition of rabbit beta-globin mRNA mediated by anti-messenger oligodeoxynucleotides covalently linked to intercalating agents. *Nucleic Acid Res.* (1995) 15(12):4717-4736.

66. SHAW JP, KENT K, BIRD J, FISHBACK J, FROEHLER BF: Modified deoxyoligonucleotide stable to exonuclease degradation in serum. *Nucleic Acids Res.* (1991) 19:747-750.

67. OTT J, ECKSTEIN F: Protection of oligonucleotide primers against degradation by DNA polymerase I. *Biochemistry* (1987) 26(25):8237-8241.

68. HOKE GD, DRAPER K, FREIER SM *et al.*: Effect of phosphorothioate capping on antisense oligonucleotide stability, hybridization and antiviral efficacy versus herpes simplex virus infection. *Nucleic Acid Res.* (1991) 20:5743-5748.

69. CORNISH KG, IVERSEN PL, SMITH L, ARNESON M, BAYEVER E: Cardiovascular effects of a phosphorothioate oligonucleotide with sequence antisense to p53 in the conscious rhesus monkey. *Pharmacol. Commun.* (1993) 3:239-247.

70. GALBRAITH WM, HOBSON WC, GICLAS PC, SCHECHTER PJ, AGRAWAL S: Complement activation and hemodynamic changes following intravenous administration of phosphorothioate oligonucleotides in the monkey. *Antisense Res. Dev.* (1994) 4:201-206.

71. HENRY SP, BOLTE H, AULETTA C, KORNBURST DJ: Evaluation of the toxicity of ISIS 2302, a phosphorothioate oligonucleotide, in a four week study in cynomolgus monkeys. *Toxicology* (1997) 120:145-155.

72. IVERSEN PL, CORNISH KG, IVERSEN LJ, MATA JE, BYLUND DB: Bolus intravenous injection of phosphorothioate oligonucleotides causes hypotension by acting as a1-adrenergic receptor antagonists. *Toxicol. Appl. Pharmacol.* (1999) 160:289-296.

73. HEDIN U, WAHLBERG E: Gene therapy and vascular disease: potential applications in vascular surgery. *Eur. J. Vasc. Endovasc. Surg.* (1997) 13:101-111.

74. KIPSHIDZE NN, IVERSEN P, KIM HS *et al.*: Advanced c-myc antisense (AVI-4126)-eluting phosphorylcholine-coated stent implantation is associated with complete vascular healing and reduced neointimal formation in the porcine coronary restenosis model. *Catheter Cardiovasc. Interv.* (2004) 61(4):518-527.

75. ZHANG XX, CUI CC, XU XG, HU XS, FANG WH, KUANG BJ: *In vivo* distribution of c-Myc antisense oligonucleotides local delivered by gelatin-coated platinum-iridium stent in rabbits and its effect on apoptosis. *Chin. Med. J. (Engl.)* (2004) 117(2):258-263.

76. PORTER TR, IVERSEN PL, LI S, XIE F: Interaction of diagnostic ultrasound with synthetic oligonucleotide-labeled perfluorocarbon-exposed sonicated dextrose albumin microbubbles. *J. Ultrasound Med.* (1996) 15:577-584.

77. MANN MJ, WHITTEMORE AD, DONALDSON MC *et al.*: *Ex vivo* gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomised, controlled trial. *Lancet* (1999) 354(9189):1493-1498.

78. KUTRYK MJ, FOLEY DP, VAN DEN BRAND M *et al.*: Local intracoronary administration of antisense oligonucleotide against c-myc for the prevention of in-stent restenosis: results of the randomized investigation by the Thoraxcenter of antisense DNA using local delivery and IVUS after coronary stenting (ITALICS) trial. *J. Am. Coll. Cardiol.* (2002) 39(2):281-287.

- **ITALICS trial result. This was the first clinical experience with local delivery of an antisense compound in coronary arteries using a local delivery catheter.**

79. HUDZIAK RM, BAROFSKY E, BAROFSKY DF *et al.*: Resistance of morpholino phosphorodiamidate oligomers to enzymatic degradation. *Antisense Nucleic Acid Drug Dev.* (1996) 6:267-272.

80. HUDZIAK RM, SUMMERTON J, WELLER DD, IVERSEN PL: Antiproliferative effects of steric blocking phosphorodiamidate Morpholino antisense agents directed against c-myc. *Antisense Nucleic Acid Drug Dev.* (2000) 10:163-176.

81. DANI C, BLANCHARD JM, PIECHACZYK M, EL SABOUTY S, MARTY L, JEANTEUR P: Extreme instability of myc mRNA in normal and transformed human cells. *Proc. Natl. Acad. Sci. USA* (1984) 81:7046-7050.

82. KIPSHIDZE N, KEANE E, STEIN D *et al.*: Local delivery of c-myc neutrally charged antisense oligonucleotides with transport catheter inhibits myointimal hyperplasia and positively affects vascular remodeling in the rabbit balloon injury model. *Catheter. Cardiovasc. Interv.* (2001) 54:247-256.

83. KIPSHIDZE NN, KIM H-S, IVERSEN P *et al.*: Intramural delivery of advanced antisense oligonucleotides with infiltrator cathetor inhibits c-myc expression and intimal hyperplasia in the porcine stent restenosis model. *J. Am. Coll. Cardiol.* (2002) 39(10):1686-1691.

84. SHEPPARD R, EISENBERG MJ: Intracoronary radiotherapy for restenosis. *N. Engl. J. Med.* (2001) 344(4):295-297.

85. HERDEG C, OBERHOFF M, BAUMBACH A *et al.*: Local paclitaxel delivery for the prevention of restenosis: biological effects and efficacy *in vivo*. *J. Am. Coll. Cardiol.* (2000) 35(7):1969-1976.

86. KIPSHIDZE NN, PORTER TR, DANGAS G *et al.*: Systemic targeted delivery of antisense with perflourobutane gas microbubble carrier reduced neointimal formation in the porcine coronary restenosis model. *Cardiovasc. Radiat. Med.* (2003) 4(3):152-159.

87. KIPSHIDZE NN, OVERLIE P, DUNLAP T *et al.*: First human experience with local delivery of novel antisense AVI-4126 with infiltrator catheter in *de novo* native and restenotic coronary arteries: six-month clinical and angiographic follow-up from AVAIL study. *Circulation* (2004) 110(17):III-757. Abstract.

- **AVAIL trial result.**

Affiliation

Nicholas Kipshidze MD, PhD, FACC[1†], Mykola Tsapenko MD, PhD[2], Patrick Iversen PhD[3] & Denis Burger PhD[4]

[†]Author for correspondence

[1]Associate Director of Clinical Research, Lenox Hill Hospital, Department of Interventional Cardiac & Vascular Services, 130 East 77th Street, New York, NY 10021, USA
Tel: +1 212 434 6835; Fax: +1 212 434 2205; E-mail: NKipshidze@Lenoxhill.net

[2]Iowa University Hospitals and Clinics, Department of Internal Medicine, Iowa City, IA, USA

[3]Senior Vice-President, AVI BioPharma, Portland, OR, USA

[4]CEO, AVI BioPharma, Portland, OR, USA